Role of testosterone in the activation of sexual behavior and neuronal circuitries in the senescent brain.

ROLE OF TESTOSTERONE IN THE ACTIVATION OF SEXUAL BEHAVIOR AND NEURONAL CIRCUITERIES IN THE SENESCENT BRAIN.

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1. THE BRAIN: A TARGET ORGAN FOR THE ACTION OF GONADAL STEROIDS.

Several studies performed in mammals and, more recently, in other vertebrates, demonstrated that sex differences in reproductive behavior as well as in neuronal circuitries involved in its control largely depend on steroid hormones. The perinatal exposure to gonadal steroids and the presence of the appropriate gonadal hormones in the adulthood are necessary for the full expression of sexual behaviour (for a complete list of references see Panzica et al., 1995). More recently, a new group of studies indicated that gonadal hormones can cause changes in brain morphology and functions in the adult brain also in regions which are not directly related to sexual functions (i.e. the regulation of cholinergic neurons by estradiol in the rat forebrain according to a sexually dimorphic pattern). Furthermore, other steroid hormones either than gonadal hormones are also effective on neural structures which do not belong to the traditional neuroendocrine brain targets (Luine and Harding, 1994). With aging, the gonads undergo anatomical, histological and vascular changes, and as a result of these changes all forms of circulating gonadal hormones decrease in both female and male. The magnitude of this decline is considerably dependent on individuals, age and health status (for a review see Timiras et al., 1995). As a consequence, alterations that occur in neuroendocrine systems during aging can provide new insights into the general problem of how steroid modulate neuronal circuitries

throughout the life.

The localization of gonadal steroid hormone sensitive regions in the brain has been accomplished in many vertebrate species. These studies established that receptor sites for estrogens, androgens, progestins, glucocorticoids, and mineralcorticoids exist in a phylogenetically stable manner in various regions of the brain (McEwen et al., 1979).

The immunocytochemical detection of steroid receptors at neuronal level of resolution gave strenght to the hypothesis that a specific action of these hormones in the differentiation and in the postnatal development of target cerebral circuits did occur. Indeed receptors for gonadal steroids and dimorphic structures were frequently observed in the same or in closely related regions, providing indirect evidence that the origin and the presence of these neuroanatomical dimorphisms might be determined by the action of gonadal steroids (for a list of reference see Kawata, 1995).

A more convincing demonstration of the significance of gonadal hormones in the regulation of sexual dimorphisms has been recently provided by the administration of antisense oligoprobes to estrogen receptor (ER). When these antisense oligoprobes were administered during a sensitive developmental period they prevent the establishment of the sexual dimorphism in the rat preoptic region (McCarthy et al., 1993).

2. EFFECTS OF GONADAL HORMONES ON BRAIN CIRCUITRIES.

The physiological effects of steroid hormones (in particular those of gonadal hormones) are generally classified, on the basis of their latency and duration, as long-term or short-term effects (Fink et al., 1991). Long-term effects last for many days, weeks (reversible effects), or even lifetime (irreversible effects). The short- term effects (always reversible) take either only minutes (or seconds) to develop (rapid effects), or much longer time (intermediate effects) and may last for several hours. The very short-term effects of steroid hormones are probably depending on a direct membrane effect (Schumacher, 1990; McEwen, 1991; Ramirez et al., 1996). Although the fast non-genomic mechanism may trigger the influence of these hormones on brain circuitries, in the present review we will focus on the slower and more permanent genomic effects.

The genomic mechanism of gonadal hormone action within nerve tissue cells (both neurons and glial elements) includes the coupling to specific intracellular receptors and the subsequent binding of the activated hormone-receptor complex to steroid receptor binding sites known as hormone response elements in the regulatory region of genes [Ramirez et al. (1996) for review]. The mechanism of the steroid action on gene expression (mostly unknown) can cause either the activation or the repression of

several genes and of the associated sequence of biochemical events. The effect of steroid on the neuronal genome may lead to several morphological and functional modifications of the brain structures. These changes include: modification of nuclear size, rearrangement of the ultrastructure of cellular compartments related to protein synthesis, changes in neuronal enzyme levels, modification of cell body size or shape, variations in the rate of synthesis of neurotransmitters or neuropeptides, alteration of the efferent circuits, sprouting of axonal projections, growth of dendritic arborization with increased number of spines, modification of afferent circuits, altered glial elements, increase in cell number, and volumetric changes of neuronal aggregations (brain nuclei).

The regulation of the number of cells in a selected region is one of the most significant mechanisms to determine long-term irreversible changes (currently called *organizational*) in the brain which are instrumental in the determination of the constitution of sexually dimorphic pathways. The variations in cell number are generally based on three different mechanisms : (1) the steroid hormone could stimulate neurogenesis in one sex, or (2) could prevent neuronal death, or (3) could regulate processes influencing the differentiation of neurons. These effects are not univocal and may differ according to the various brain regions (Arnold and Schlinger, 1993).

Long-term reversible effects of steroids on neural pathways (generally described as *activational* effects) occur in the adulthood regardless the sexual differentiation of the involved circuitries. [for reviews see Breedlove (1992); Panzica et al. (1995)].

Morphological changes in the brain due to the action of gonadal hormones do not require hormone involvement at every point. For example the stimulation of axonal growth (under the control of sex steroids) could stimulate the growth and differentiation of the target region which in itself is lacking of gonadal hormone receptors (domino theory: (Arnold and Schlinger, 1993). Therefore, the morphology and connections of a specific brain region is the result of an interaction of hormone-dependent and hormone-independent entities (Tobet and Fox, 1992).

Furthermore it is important to underline that steroid hormones can act on nervous tissue also through their metabolites. For example, testosterone (T) can be actively metabolized within the brain into a number of androgenic or estrogenic steroids. Two metabolic pathways of T are particularly relevant to the control of

male sexual behavior: 5⟨-reduction and aromatization. The 5⟨-reductase

catalyzes the reduction of T into 5⟨-dihydrotestosterone (5⟨-DHT), a steroid that binds with high affinity to the androgen receptor (Celotti et al., 1992). The enzymatic complex aromatase (ARO) catalyzes the aromatization of T producing

17®-estradiol(E_2). Estrogens bind with high affinity to ER and in this way activate physiological and behavioral responses that are different from those activated by androgens (Balthazart, 1993; Roselli and Resko, 1993).

3. SEXUALLY DIMORPHIC STRUCTURES IN THE VERTEBRATE BRAIN.

Sexually dimorphic nuclei or regions have been described in several species of tetrapods and have been observed in different contexts including:

1. neuronal groups directly related to peripheral dimorphic organs (i.e. motoneurons of the spinal cord controlling the penis muscles or motoneurons controlling the avian syringeal musculature);

2. cerebral nuclei without any evident connection to sexually dimorphic structures or organs (i.e. the dimorphic nuclei of the preoptic and limbic regions in many vertebrate species including humans, the ventromedial hypothalamus, or the accessory olfactory pathway in the rat).

In many cases these sexually dimorphic structures are under the control of steroid hormones also during adulthood to maintain their sexually dimorphic characteristics. A complete revision of the literature concerning the sexually dimorphic structures that have been described in the vertebrate brain and their regulation by gonadal hormones is out of the aim of the present review. For further details and a complete list of references, the reader is addressed to some recent reviews (Tobet and Fox, 1992; Breedlove, 1992; Panzica et al., 1995).

It is interesting to note that, apart from motorneurons controlling dimorphic muscles, only in a few cases the sexually dimorphic structures have been directly related to sexual behavior. Since male sexual behavior is differentiated in many species (it is more easily activated by androgens in males than in females), it could be expected that sexually dimorphic structures would play a key role in the activation of this behavior. Moreover lesion and hormone implantation studies demonstrated that the preoptic region plays a key role in controlling copulatory and lordosis behaviors (Meisel and Sachs, 1994; Pfaff et al., 1994). However, it has been difficult to relate in a causal manner the sexually dimorphic nucleus observed in the rat preoptic area with the mechanism mediating male copulatory behavior (Arendash and Gorski, 1983; De Jonge et al., 1990). The same conclusion was reached in other mammalian species studied so far except in gerbils, in which the pars compacta of the medial preoptic nucleus was demonstrated to play a key role in the control of male sexual behavior (Yahr and Gregory, 1993). In humans there are studies that compared gender and sexual attitudes, demonstrating that some preoptic and limbic nuclei have volume values more similar to the psychological than to the genetic sex, but of course no experimental work has been done on this theme (Le Vay, 1991; Swaab et al., 1992; Zhou et al., 1995).

In birds two models have been investigated, one is that of the song controlling nuclei of oscine (singing birds, Arnold, 1990), and the second one is the medial preoptic nucleus (POM) of the Japanese quail (Panzica et al., 1996c). This last structure is a sexually dimorphic nucleus representing a key center in the action of steroids on male sexual (copulatory) behavior (Balthazart and Surlemont, 1990). Various aspects of the POM morphology change in relation to circulating gonadal hormones and therefore, the structure of this nucleus provides a model for the action of steroids in the brain. Several forms of neuronal plasticity are observed in the POM and this nucleus constitutes an ideal model for the study of sexual behavior in a context that permits a functional interpretation of the changes observed at the cellular level. In a following section we will therefore describe in details this model that has been also widely employed in researches concerning the neuroendocrinology of reproduction, as well as of aging [for a complete list of references see Panzica et al. (1996c)]

4. THE JAPANESE QUAIL BEHAVIORAL MODEL

Testosterone (T) is the molecule inducing the full expression of male copulatory behavior in quail, but, in contrast to what is observed in rodents, this behavior shows a quite exteme sexual dimorphism in this species: in laboratory test conditions, sexually mature males almost never fail to exhibit the complete copulatory sequence (grabbing, mount attempts, mounts, and cloacal contact movements). They disappear after castration and they appear when castration is followed by cronic T-treatment in male. Conversely, high doses of T on either intact or gonadectomized females are not sufficient to activate male behavioural sequence. On the contrary, the female-type receptive behavior can be activated in both the sexes by an appropriate treatment with estrogens. The sex difference in sensitivity to the activating effects of T on copulatory behavior results from the early exposure of female brain to higher levels of circulating estrogens. This evidence indicates that the neuronal circuits supporting male reproductive behavior are precociously sexually differentiated in this species [major details on these experiments are discussed elsewhere (Balthazart and Foidart, 1993; Panzica et al., 1996c)].

The activation of copulatory behavior in castrated male quail can be obtained by treatment with high doses of estrogens or by a combined treatment with

physiological doses of E_2 and 5⟨-DHT. This suggests that, in physiological conditions, although T is the most effective steroid on copulatory frequencies both the androgenic and estrogenic product of T metabolism are responsible for the T- dependent behavioral activation. On the other hand, ARO inhibitors can

completely suppress the effects of T on copulatory behavior, while 5⟨-reductase inhibitors have little or no effect. This observation demonstrates that the aromatization of T is a limiting step for the activation of sexual behavior by T in male quail to occur (Balthazart and Foidart, 1993). Therefore, studies on the

location and response of the ARO-producing system are integral to understanding how the system works and what may become altered during aging.

5. NEURONAL CIRCUITS CONTROLLING MALE COPULATORY BEHAVIOR IN QUAIL.

In the course of studies analyzing the dimorphic mechanisms involved in the activation of sexual behavior we discovered a specialized region (the POM) of the POA showing an evident dimorphism in the volume: the POM is significantly larger in adult male than in adult female quail (Viglietti-Panzica et al., 1986). Its volume is also steroid-sensitive in adulthood: it decreases when circulating levels of testosterone are low (castration, exposure to short-days) and it increases when testosterone levels are high (treatment with testosterone, exposure to long-days) (Panzica et al., 1987, 1991). The POM is a necessary and sufficient site of steroid action for the activation of male copulatory behavior (Balthazart and Surlemont, 1990). Cytoarchitectural and morphometrical studies provided evidence for the existence of two neuronal populations, located in the medial and in the dorso- lateral portions of the POM and distinguished by their size. These two populations are characterized by different sensitivities to T in adulthood. The medial population appears smaller and relatively insensitive to T in both sexes, whereas the dorso-lateral population (only in males) changes in size in correlation to the levels of T. The size of neurons in the dorsolateral part of POM appears to be irreversibly affected by embryonic exposure to steroids and this feature is therefore a good correlate of the behavioral sex difference (Aste et al., 1991; Panzica et al., 1991). Ultrastructural studies suggest that T influences specific cellular compartments involved in the synthesis and processing of proteins (Panzica et al., 1996a). The POM is characterized by the presence of a wide variety of neurotransmitters, neuropeptides and receptors [for a complete list of references see Panzica et al. (1992, 1996c)]. It can, in addition, be specifically distinguished from the surrounding POA by the presence of ARO-ir cells

(Balthazart et al., 1990b), by high density of $\langle_2$-adrenergic receptors (Ball et al., 1989), and by a denser vasotocinergic innervation (Viglietti-Panzica et al., 1994). Some of these neurochemical markers of the dimorphic nucleus are themselves modulated by steroids. In particular, the ARO-ir cells of the lateral POM appear to be a key target for steroids in the activation of male copulatory behavior. The POM is bidirectionally connected to many brain areas (Balthazart et al., 1994). It receives inputs from a variety of sensory areas and fregulatory areas (i.e. catecholaminergic cell groups). This nucleus also sends outputs to “neurovegetative” centers and to brain regions directly connected to the motor pathways. These connections fully support the role of the POM as an integrative center for the control of male sexual behavior. The available data indicate that there is a high degree of steroid-induced neuronal plasticity in the POM including changes in neuronal function, in protein synthesis and in specific inputs. These phenomena are also directly related to a clear functional output, the activation of male sexual behavior (Panzica et al., 1996c).

5.1 - Aromatase Activity and Aromatase-Immunoreactive Cells

Biochemical studies characterized and localized the enzyme aromatase in the quail brain, showing that a high level of ARO activity is present in the quail POA. Further studies employing microdissections by the "Palkovits" punch technique demonstrated that all the ARO activity of the POA was localized within the POM [for a review on these data see Balthazart et al. (1990a)].

Four main groups of ARO-ir cells are observed by immunocytochemistry, namely in the POA, the septal region, at the level of the nucleus of the stria terminalis (nST), and in the ventromedial hypothalamic nucleus. All preoptic ARO-ir cells are localized within the POM; the dense cluster of ARO-ir cells is preciselly associated to the nucleus throughout its rostral-caudal extent.

ARO-ir cells represent a large fraction of the total neurons present in the POM (about 40% of the total in the lateral POM; 20% in the medial part of the nucleus (Aste et al., 1994)). Only a small portion of this population (about 20%) show also immunopositivity for ER (Balthazart et al., 1991; Dellovade et al., 1995).

5.2 - Vasotocin (VT) immunoreactive system.

The distribution of VT-ir cells and fibres has been studied in several avian species and the characteristics of the vasotocinergic system has been recently reviewed (Viglietti-Panzica and Panzica, 1991; Jurkevich et al., 1996a). In quail, the majority of large VT-ir or VT-gene expressing cells (Viglietti-Panzica, 1986; Aste et al., 1996a) is located laterally or periventricularly in the preoptic and anterior hypothalamic regions, however a specific parvocellular cell group (located in the nST) has also been observed (Aste et al., 1995, 1996c). VT-ir fibers are observed in several intra- and extrahypothalamic regions involved in the control of reproduction (Jurkevich et al., 1996a), as the POM (control of copulatory behavior), the lateral septum [site of the largest population of neurons producing gonadotropin-releasing hormone (GnRH) in birds], and nucleus intercollicularis (ICo, a mesencephalic center controlling vocalizations). In a recent study we revealed the existence of widespread anatomical interactions between estrogen-synthesizing neurons and vasotocinergic fibers in the quail brain. Close relationships between VT-ir fibers and ARO-ir cell bodies or processes were in fact observed (Balthazart et al., 1997). These data suggest that VT may be involved in the regulation of several aspects of reproduction in birds.

5.3 - Effects of gonadectomy and testosterone therapy on sexually differentiated neuronal circuitries.

ARO activity, ARO-ir elements, VT-ir innervation of the POM and lateral septum, and VT-ir or VT gene expressing elements of the nST show a sexually dimorphic distribution pattern in the quail brain.

These systems were therefore investigated in the male quail to understand whether gonadectomy and T replacement therapy could influence their distribution pattern, or the production and storage of the involved neurochemical markers. A detailed description of all the experiments done on this subject has been recently published [for the complete review of the literature see Panzica et al. (1996c)], we will try to summarize here the most important results.

ARO synthesis is strongly dimorphic and is stimulated by circulating T only in the male (Schumacher and Balthazart, 1986). In the POM, a loss of ARO-ir cells coincident with the loss of male sexual behavior was observed in young castrated males. The decrease in the number of ARO-ir elements was presumably linked to the drop in the levels of available T since subcutaneous administration of T by means of silastic implants restored aromatase staining in castrate males. These data demonstrate that sexual behavior and ARO-immunoreactivity show parallel changes in young male quail (Balthazart et al., 1992). As already mentioned, in male quail ARO-ir cells are more numerous in the dorso-lateral than in the medial part of the POM. The number of lateral ARO-ir cells decreases in castrated males to 10 percent of the initial number. On the contrary, the positive neurons in the medial part of the POM are reduced only to 30 percent of their initial number (Aste et al., 1994). These results suggest hence the presence of two types of aromatase cells in the quail POA in young male bird. A small population of ARO- ir cells is present in the brain independently of the steroid environment and this represents at least one third of the cells that are found in the medial part of the POM. Other larger ARO-ir cells are sensitive to steroid stimulation and they almost completely disappear in castrated birds. Most of the observed effects of T on ARO-ir cells could be reproduced by treatment with either E_2 alone or E_2 in

combination with 5⟨-DHT (Aste et al., 1994).As already mentioned, the VT-ir system in the nST is sexually dimorphic in

galliforms as was recently demonstrated by means of immunocytochemistry and *in situ* hybridization studies (Aste et al., 1996c; Jurkevich et al., 1996b). In mammals, several studies have elucidated the role of the nST and of other limbic structures (the medial amygdaloid nucleus) as source of the majority of extraneurohypophyseal sexually dimorphic T-dependent vasopressinergic projections [i.e. the projection to the lateral septum, for a review see (De Vries et al., 1994)]. Released directly into the brain interstitial fluid vasopressin (VP) derived from limbic structures may account for its own neuromodulator and/or neurotransmitter-like effects including regulation of sex specific behavior (De Vries, 1990). Sexually dimorphic VT-ir fibers were also observed in quail, specifically in the lateral septum (Viglietti-Panzica et al., 1992), in the POM (Aste et al., 1996b), and in the ICo (unpublished results). Specific experiments performed on intact, castrated, and castrated plus T young male quail have demonstrated that in POM and lateral septum the extent of vasotocinergic innervation is strictly dependent by circulating T levels and parallels the changes in sexual behavior (Viglietti-Panzica et al.,

1992, 1994; Aste et al., 1996b). Very recent experiments have finally demonstrated that VT is exerting an inhibitory role in the expression of male quail sexual behavior when peripherally or intracerebroventricularly injected (Castagna et al., 1996).

6. AGING IN THE JAPANESE QUAIL

6.1 - Behavioral aspects

There is a large variability in the sexual behavior of old male quail. Some individuals become spontaneously sexually inactive (senescent) from the age of 18 months. Plasma levels of androgen decrease in all middle-aged birds (18-30 months of age) and do not differ significantly between active and inactive males. Once the animal has become completely senescent, the testes cease both spermatogenesis and steroid production. The senescence is typically observed very late in aging (after 30 months or older). At this time, plasma androgen levels are in concert with reproductive status (Ottinger, 1992; Ottinger et al., 1995). At this time few individuals still retain sexual activity and they have larger testis and increased levels of circulating androgens (see Fig. 1A-C).

These data suggest that the simplistic idea that the loss of gonadal steroids triggers the process of behavioral reproductive aging in the male Japanese quail is contradictory. Similar to data from mammals, plasma androgen levels do not significantly change during aging until the male has become reproductively senescent (Ottinger, 1992). In both rats and quail, aging males that remain sexually active retain higher plasma androgen levels and these levels are independent from the intensity of sexual activity. More intriguing is the fact that the sexual behavior can be restored in senescent male quail by administration of exogenous T (Ottinger and Balthazart, 1986), whereas mammals do not fully respond to this treatment (Taylor et al., 1996). If we assume that sexual behavior is primarly “written” in neuronal circuiteries, this means that compared to mammals, quail maintain a neuronal machinery that retains the capability to respond, even in aging. It has to be stressed however that the dose of T used to stimulate sexual behavior in senescent males was double the effective dose for young or in adult birds. This suggests that part of the machinery does deteriorate during aging and the recovery of function may be based on a certain degree of plasticity still remaining in the old quail brain, or to on an overstimulation of the persisting system. Hence, we have been investigating the neuroendocrine systems affected by the process of aging and which specific systems are involved in the recovery of behavior in these old senescent males.

6.2. Neuronal circuitries

6.2.1. The ARO-ir system. Age-related changes in this system were studied in 3 groups of quail: 6 month-old adult sexually active males, 36 month-old senescent males, and 36 month-old sexually

active males (Dellovade et al., 1995). In old senescent males the decrease in the number of ARO cells was significant compared with the other two groups. Old active males had an intermediate number of ARO-ir cells as compared with the other 2 groups (see Fig. 1D). A significant decrease of the immunoreactive population which is located in the nST was observed only in old senescent males. An interesting finding was that the percentage of neurons co-expressing ER increased with age, rising from the 19% of adult birds to 25% of old inactive male quail. This means that the reduction in the number of ARO-ir cells is primarily interesting those elements that do not show ER. These data confirmed hence the importance of the ARO-ir system for the activation of sexual behavior: old active males show at the same time sexual behavior, larger testis, and a number of ARO-ir neurons higher than old inactive birds. A more detailed morphometrical study (Panzica et al., 1994) demonstrated that in contrast to young castrated males, the ARO-ir loss in old males (both active and inactive) was more marked in the medial vs the lateral POM In old senescent males the decrease in number was paralleled by a similar decrease in the cell size, indicating a loss in synthetic structures similar to young castrated males . In old active males the ARO-ir cells were more numerous in the lateral than in the medial POM and had a cell size which was significantly larger than in the adult sexually active and old inactive males. Two conclusions can be drawn from these experiments: a) the ARO system is specifically influenced by the reduction of circulating T both in the young and in the old animal, and loss of part of this neuronal system resulted in a loss of the sexual performances; b) the specific mechanisms that the hormone decline provokes on the ARO system may vary in the young and in the old male quail. Moreover, the increase in cell size observed in old sexually active males has been already observed in other neurochemically identified systems during aging and it is generally considered as a signal of overstimulation of the surviving portion of the system which is trying to compensate for the partial loss of cells (Brody, 1992). Accordingly, it can be considered as a compensatory effect to minimize the deficient production of aromatase (due to the reduction in number of these elements) in order to maintain a production of E_2 sufficient to induce sexual activity .

6.2.2. The vasotocin system In recent studies we demonstrated an age-related decrease in VT-immunoreactivity in several brain areas (POM, lateral septum, and nST) important for the regulation of both endocrine and behavioral aspects of reproduction (Panzica et al., 1996b). Other portions of the VT-ir system (i.e. the magnocellular system) do not show the same decrease in positive structures (Viglietti-Panzica et al., 1996). Further, this age-related decrease in VT- immunoreactivity was restored to levels similar to those observed in adult male quail by T replacement therapy, a treatment that restores the sexual behavior in old senescent males (Fig. 1E-F). Similarly to what previously described for young males (Viglietti-Panzica et al., 1992, 1994) we have confirmed in the aged birds a direct correspondence between VT innervation of septo-preoptic region and sexual behavior. The decline of sexual behavior induced by castration or photoregression in the young male or by physiological events in old senescent males is paralleled by a decline in VT-immunoreactivity in specific cerebral areas.

Moreover, these data indicate that the VT system retains a high degree of plasticity in old male quail. The source of the VT innervation of POM and lateral septum is still unknown in quail. However, the sexually dimorphic population of weakly immunostained cells located in the nST (showing itself a T-dependent decrease in the immunostaining during aging) represents a very good candidate as a source of this innervation.

A similar plasticity was observed in the mammalian VP system. A decrease in VP-ir fibers is present during aging and subcutaneous implants of T in 33 month- old Norway male rats restored VP innervations in sex steroid sensitive regions (Goudsmit et al., 1988), however, in this case, no behavioral recovery has been described.

6.2.3. The gonadotropin-releasing hormone (GnRH) system The process of reproductive senescence is ultimately leading to a complete loss of both endocrine and behavioral components of reproductive function. The study of age-related changes in the GnRH system is obviously central to the comprehension of variations in the endocrine regulation of reproduction. Moreover, several studies have suggested a direct involvement of GnRH in the control of sexual behavior (Dornan and Malsbury, 1989). In rats, hypothalamic GnRH is reduced in old sexually inactive males as compared to old sexually active males (Dorsa et al., 1984). Aged rats had a decreased number of neurons expressing GnRH mRNA compared to young males (Gruenewald and Matsumoto, 1991) and morphological alterations of the synaptology of GnRH neurons (Witkin, 1989).

No morphological data are at moment available on this system in aged birds, however, several endocrinological studies have been performed on both young and aged male quail [for a review see Ottinger et al., (1997)]. GnRH concentrations decrease in the preoptic-septal area and in the median eminence to very low levels in the senescent male. Moreover, the loss of GnRH occurred first in the preoptic-septal region and then later in the median eminence, perhaps indicating the loss of synthetic capability and then later depletion of stored hormone. The GnRH release by longitudinal hypothalamic slices (containing a large portion of the GnRH system) from old males was consistently lower than from young males. There was a qualitative difference in GnRH release in slices taken from young reproductive and old inactive males with reduced amplitude of release. Lastly, slices from young reproductive males are strongly responsive to catecholaminergic stimulation of the GnRH secretion, whereas slices from old senescent males were significantly less responsive. These data provide evidence that the GnRH system is capable of response even in senescent males, but the qualitative response becomes altered during aging.

Even if direct experiments on the senescent male quail have not been performed it has to be considered the fact that E_2 stimulates not only the sexual behavior, but also, directly, the GnRH production (Ottinger et al., 1997). Therefore, changes in the ARO-producing system are probably integral to

alterations of GnRH system during aging. Moreover, immunocytochemical studies on serial sections demonstrated that VT-ir fibres have a spatial distribution very close to that of the septal group of GnRH-ir neurons (Panzica et al., 1992). These observations suggest that the VT-ir system might have a primary role in regulating the activity of GnRH neurons in quail.

7. CONCLUSION

Data reviewed in this paper demonstrate that the aging brain still maintains a certain degree of plasticity when stimulated by exogenous supply of testosterone. Moreover, in the quail model, the sensitivity of neuronal circuitries to T is paralleled by a recovery of copulatory behavior to levels comparable to those observed in adult sexually mature male quail.

Male copulatory behavior declines during aging and ceases in old senescent males. Old senescent male quail (36 months) have in fact greatly reduced plasma levels of T, Moreover, decreased levels of T, loss in testis weight and loss of behavioural capacity have been observed in photoregressed and castrated young males. However, it is not clear whether the mechanisms underlying loss of male quail sexual behavior are the same for these three conditions.

Several morphological and biochemical features of the quail brain are modified by the reduction in circulating levels of T resulting from aging, exposure to short day or surgical gonadectomy. In most of cases these modifications are complementary to the loss of sexual behavioural.

The VT-ir system shows similar changes in the young and in the old male quail. The decrease in VT-ir structures in the lateral septum, POM, and nST depends on the loss of T due to gonadectomy, exposure to short-day photoperiod (young birds) or aging process (old senescent individuals). When the birds (old and young) are supplied with exogenous T a full recovery of the immunoreactive structures is observed in all the mentioned regions. Data obtained in quail are very similar to those reported for the VP system in rat (Goudsmit et al., 1988). It should be noted that both in rodents (Fliers et al., 1985) and in quail (Viglietti- Panzica et al., 1996) the magnocellular system is not depressed by aging. On the contrary the cell size is slightly increased suggesting an activation of the system. Only the structures that are present in sex steroid dependent areas show a significant decrease in VT/VP immunoreactivity during aging. It appears hence that the VT (or VP) system in itself should not be considered the direct responsible of the loss of sexual behavior which starts when circulating levels of T are still high (and hence presumably still stimulating the system).

A loss of ARO-ir cells in the POM presumably linked to decreased levels of available T was observed in both old and young (castrated or photoregressed) males. However, this loss was more marked in young castrated than in old senescent males, and the two different populations of ARO-ir elements

which are present in the POM (lateral vs medial) were affected in opposite directions in the young and in the old quail. We have not data concerning the ARO system in old senescent birds treated with exogenous T, however we investigated the distribution of this system in spontaneusly active old birds which showed rather high levels of circulating T. The ARO-ir system of these birds is different (in terms of number of cells) from that of both the young and the old senescent males, moreover, the reported increase of the cell size suggests that the surviving portion of the system was trying to recover the functions (presumably the production of E_2) of the whole ARO system. Only a few studies have considered changes in brain aromatase activity during aging in mammals, and no immunocytochemical study has been performed. ARO activity decreases with age, and can be restimulated by supply of exogenous T (Chambers et al., 1991). This treatment is not fully active in recovering the sexual function in old rat (only the 25% of experimental old rats demonstrated ejaculation).

The peculiar situation of the quail, in which exogenous T can re-stimulate the presence of sexual behavior in very old males could be related to the peculiarity of the brain circuitery which is involved in the control of male copulatory behavior in this species. VT-ir fibers are innervating the POM and the ARO-ir cells (that play a key role in the control of sexual behavior), and the septal region where a large cluster of GnRH neurons is present. The specific way of interaction between ARO and VT and its functional implications remain unclear at present. It is, however, evident that some of the functional outputs of the brain are affected both by estrogens and VT. For example, appetitive and consummatory aspects of male sexual behavior in quail are activated by estrogens, but inhibited by the central action of VT that probably plays a tonic inhibition on these behaviors (Castagna et al., 1996).

In conclusion, it may be speculated that the sensitivity of the VT system to T (lasting also in the aged animal) could indirectly determine the restoration and regulation of the functions of the ARO system in the POM and of the GnRH system. If this is the case, the putative center of origin of these VT fibers (the nucleus of the stria terminalis) should be regarded as the most important regulatory center of the sexual behavior in quail.

ABBREVIATIONS

5⟨-DHT: 5⟨-dihydrotestosterone, AR: androgen receptor, ARO: aromatase, E_2: estradiol, ER: estrogen receptor, GnRH: gonadotropin-releasing hormone, ICo: nucleus intercollicularis, ir: immunoreactive, nST: nucleus of the stria terminalis, POA: preoptic anterior region, POM: nucleus preopticus medialis, T: testosterone, VP: vasopressin, VT: vasotocin.

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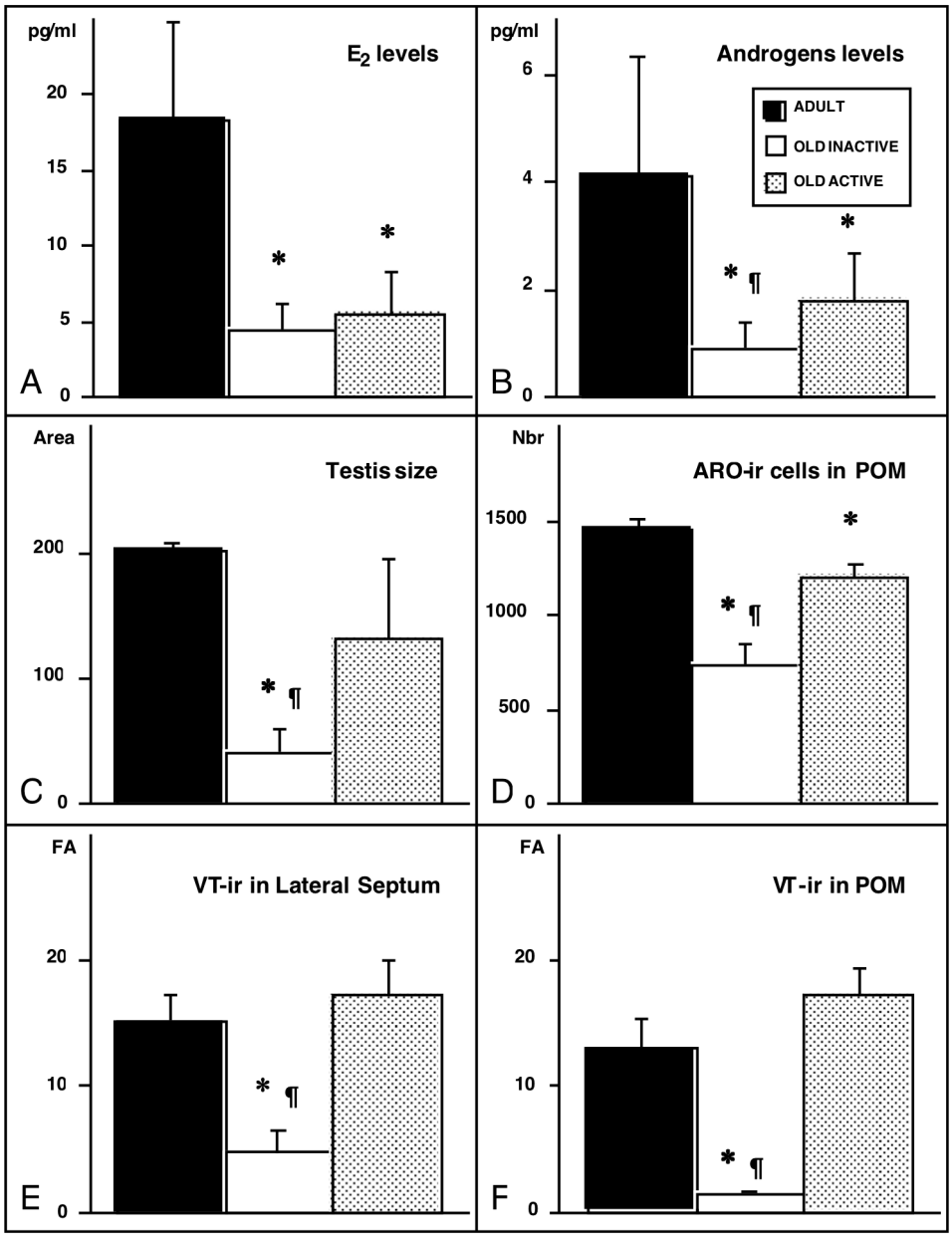
Legend

Figure.1. Histograms representing changes in endocrine, morphological, and neuronal characteristics during reproductive aging in male quail. **A-D** Old active males, are 36 month old male quail spontaneusly active; data are from Dellovade et al. (1995). **E-F** Old active males are 36 months senescent males treaed for 2 weeks with silastic implants of testosterone; data are from Panzica et al. (1996b). **A-B** Average levels of plasma estradiol (E2) and androgens in the 3 considered groups. Androgens in old active males are significantly lower than in adult, but significantly higher than in old inactive male quail. **C**. Changes in testis size. Old inactive males have a significant lower area of testis in comparison with adult and old active males. **D**. Changes in the number of aromatase immunoreactive (ARO-ir) neurons within the POM during aging. Old active males have significantly less positive cells than adult, but significantly more positive cells than old inactive males. **E-F**. Vasotocin immunoreactive (VT-ir) fiber density in lateral septum and POM. Old inactive males have significantly less fractional area (FA) covered by immunopositive structures. * statistically significant difference ($p<0.05$) in comparison to adult group; ¶ statistically significant difference ($p<0.05$) in comparison to old active group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